SECTION 7

SPECIAL TECHNIQUES

7.1 Flesh Tainting (Flavor Impairment)

7.1.1. Sublethal concentrations of chemicals, such as phenols, benzene, oil, and 2,4-D, are often responsible for imparting an unpleasant taste to fish flesh, even when present in very low concentrations.

7.1.2 Specific methods have been developed by Thomas (1969), APHA (1992), and ASTM (1992) in which untainted fish are placed in cages or exposure tanks upstream and downstream or in the laboratory from suspected waste water sources. The techniques in these references and Subsection 7.1.3 should successfully relate the unacceptable flavor produced in exposed native fish to a particular waste source.

7.1.3 The following procedures are presented as a working guide for fish flesh tainting or flavor impairment.

7.1.3.1 To ensure uniform taste quality before exposure, all fish are held in pollution-free water for a 10 day period. After this period, a minimum of three fish are cleaned and frozen with dry ice as control fish. Test fish are then transferred to the test sites, and a minimum of three fish are placed in each portable cage. The cages are suspended at a depth of 0.6 meter for 48 to 96 hours.

7.1.3.2 After exposure, the fish are filleted, frozen on dry ice, and stored at $0^{\circ}C$ until tested. The control and exposed samples are shipped to a fish tasting panel, such as is available at the food science and technology departments in many major universities, and treated as follows: (1) The fish are washed, wrapped in aluminum foil, placed on slotted, broiler-type pans, and cooked in a gas oven at $218^{\circ}C$ ($400^{\circ}F$) for 23 to 45 minutes depending on the size of the fish; (2) Each sample is boned and the flesh is flaked and mixed to ensure a uniform sample; (3) The samples are served in coded cups to judges. Known and coded references or control samples are included in each test. The judges score the flavor and desirability of each sample on a point scale. The tasting agency will establish a point on the scale designated as the acceptable and desirable level.

7.2 Fish Kill Investigations

7.2.1 Fish kills in natural waters, though unfortunate, can in many instances indicate poor water quality and environmental health leading to investigations which may improve the water quality. Prompt investigations should be organized and conducted so that the resultant data implicates the correct cause. Fish kills tend to be highly controversial, usually involving the general public as well as a number of agencies. Therefore, the investigator(s) can expect his finding to be disputed, quite possibly in a court of law.

7.2.2 Possible Fish Kill Sources

7.2.2.1 Fish mortalities result from a variety of causes, including natural and man-induced. Possible natural fish kills are caused by phenomena such as acute temperature change, storms, ice and snow cover, decomposition of natural organic materials, salinity changes, spawning mortalities, and parasitic, bacterial, and viral epidemics. Man-induced fish kills may be attributed to municipal or industrial wastes, agricultural activities, and water manipulations.

7.2.2.2 Winter kills occur in northern areas where ice on shallow lakes and ponds becomes covered with snow, and the resulting opaqueness stops photosynthesis. The algae and vascular plants die because of insufficient light, and plant decomposition results in oxygen depletion. Oxygen depletion and extreme pH variation can also be caused by the respiration or decay of algae and higher plants during summer months in very warm weather. Fish kills resulting from such causes are often associated with a series of cloudy days that follow a period of hot, dry, sunny days. Fish kills also occur in rivers below high dams immediately following the opening of a gate permitting cold hypolimnionic water to flow into the streams as in the Tennessee Valley Authority (TVA) region.

7.2.2.3 Temperature changes, either natural or the result of a heated water discharge may result in fish kills. Long periods of very warm, dry weather may raise water temperatures above lethal levels for sensitive species. A wind-induced seiche may be hazardous to certain temperature sensitive, deep-lake, cold-water fish, or fish of shallow coastal waters. Lake water inversion during vernal or autumnal turnover may result in toxic materials or oxygen-free water being brought to the surface. Interval seiche movement in which a toxic or low dissolved oxygen hypolimion flows up into a bay or bayou for a limited period of time, and later returns to normal levels may also cause fish kills.

7.2.2.4 Disease, a dense infestation of parasites, infection from bacteria, or viruses, or natural death of weakened fish at spawning time must always be suspected as contributory factors in fish mortalities.

7.2.2.5 Occasionally fish may be killed by toxins released from certain species of living or decaying algae that reached high population densities because of the increased fertility resulting from organic and inorganic pollution.

7.2.2.6 Investigations in Tennessee have shown that the leaking of small amounts of very toxic chemical from spent pesticide-containing barrels used as floats for piers and diving rafts in lakes and reservoirs can produce extensive fish kills (TVA, 1968).

7.2.2.7 Industrial waste discharges and waste discharges from a municipal or domestic type sewerage system may be potential sources of fish kills. These wastes may be subjected to treatment of a municipal treatment plant or may be discharged directly, untreated, to a stream. Generally, the municipality or owner of the sewerage system is held responsible for any discharge in such a system; consequently, after collecting samples, the owner or a representative of the owner of the sewerage system should be contacted. This may be a sewage treatment plant operator, city engineer, public works supervisor, a subdivision developer, etc. If the cause of the fish kill was the result of an industrial waste discharge to a municipal sewer and thence to a stream, information should be obtained from a municipal official about the industry and the problem. This should be done only in cooperation with a municipal official.

7.2.2.8 Pollution capable of causing fish kills may result from such agricultural operations as pesticide dusting and fertilizer applications, as well as manure or other organic material discharges to a stream. Generally, fish kills related to these factors will be associated with rains and runoff. The source or type of pollution may be difficult or impossible to locate exactly because it may involve a large area. Talking to local residents may help pinpoint the specific problem area. Runoff from fields, drainage ditches, and small streams leading to the kill area are possible sampling places which may be used to trace the causes.

7.2.2.9 Temporary or intermittent activities, such as mosquito spraying, construction activities involving chemicals, other toxic substance, and herbicide containing materials toxic to fish such as arsenic, are also potential causes of fish kills. As with agricultural activities, tracing the cause of these kills is difficult and may require extensive sampling. Accidental spills from ruptured tank cars, pipelines, etc., and dike collapse of industrial pond dikes are frequently sources of fish kills.

7.2.3 Types and Extent of Fish Kills

7.2.3.1 One dead fish in a stream may be called a fish kill. However, in a practical sense some minimal number of dead fish observed plus additional qualifications should be used in reporting and classifying fish kill investigations (USEPA, 1973). These qualifications are based on a stream approximating 200 feet in width and 6 feet in depth. For other size streams, adjustments should be made.

7.2.3.2 Minor fish kills (1-100 dead or dying fish) may be considered "no fish kill" if confined to a small area or stream reach provided this is not a recurring event. For example, fish kill occurring near a waste water outfall in which stream dilution mitigates the effect of the deleterious material. If this is a recurring situation, it could be of major significance and should be investigated.

7.2.3.3 Moderate fish kill (100 - 1000 dead or dying fish) may be considered to have occurred if a number of species and individuals habe been affected in 1-2 km of stream where dilution would have been expected to play a mitigating role. Apparently normal fish may be collected immediately downstream from the observed fish kill area.

7.2.3.4 Major fish kill (1000 - 10,000 fish or more dead or dying fish) may be considered to have occurred in 10-20 km of a stream in which dilution would have been expected to have a mitigating effect and when many species of fish are affected and dying fish may still be observed downstream.

7.2.4 Preparation for Field Investigation

7.2.4.1 All possible speed must be exercised in conducting the initial phases of any fish kill investigation because fish disintegrate rapidly in hot weather, and the cause of death may disappear or become unidentifiable within a short period of time. Success in solving a fish kill problem is usually related to the speed with which investigators can arrive at the scene after a fish kill begins. The speed of response in the initial investigation is enhanced through the training of qualified personnel who will report immediately the location of observed kills, the time that the kill was first observed, the general kinds of organisms affected, an estimate of the number of dead fish involved, and any unusual phenomena associated with the kill.

7.2.4.2 Because there is always the possibility of legal liability associated with a fish kill, lawyers, judges, and juries may scrutinize the investigation report. Therefore, the investigation must be made with great care. When investigating a fish kill, a specific litigation or case number should be assigned and used on all labels, field data sheets, photographs, and other records related to the fish kill investigation. Table 1 is a general flowchart to help with the coordination of a fish kill investigation.

7.2.5 Legal Aspects

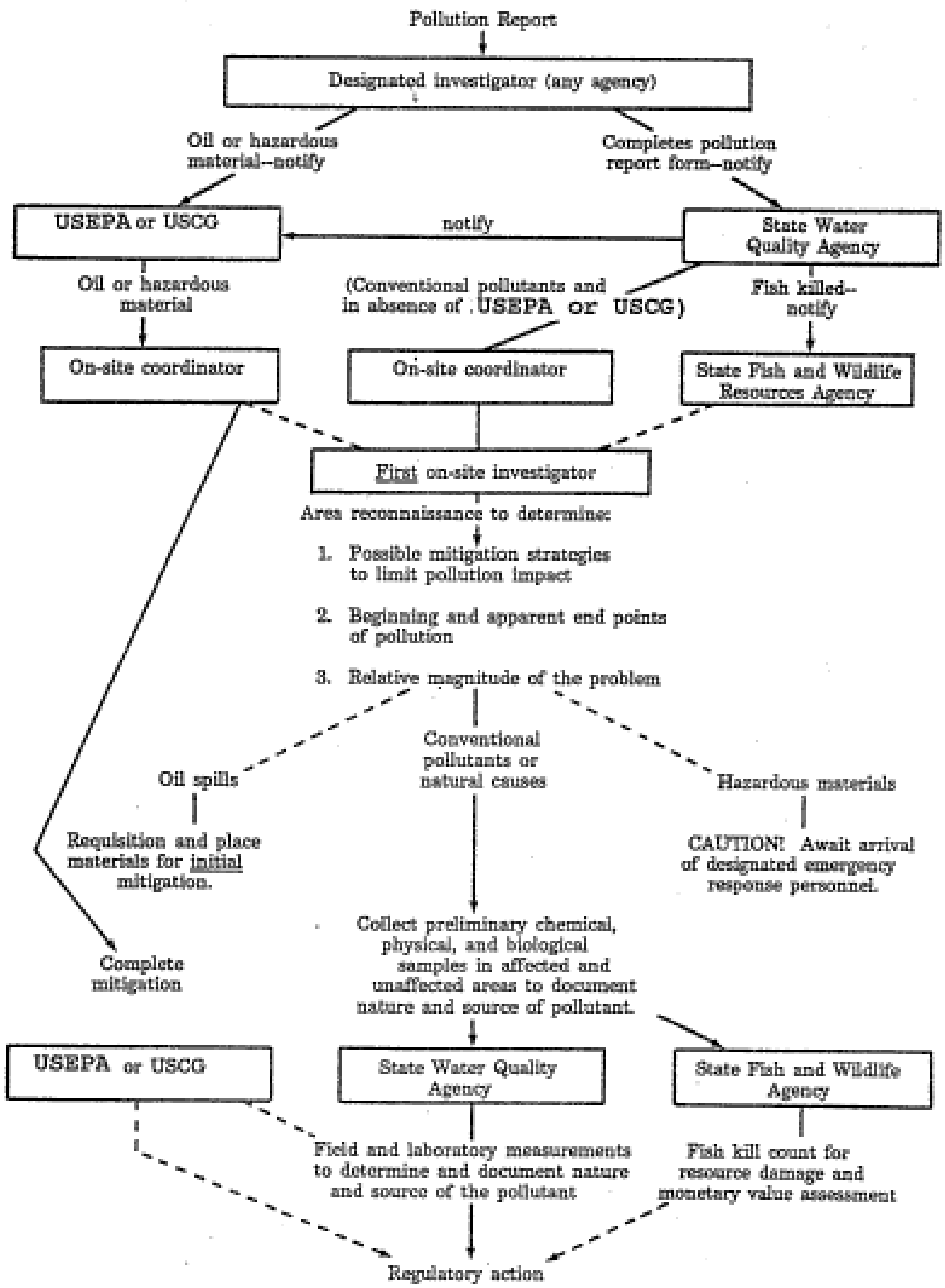
7.2.5.1 A chain-of-custody (see Section 2, Quality Assurance and Quality Control) must be adhered to when any fish kill is investigated and samples collected for analysis and presentation as evidence. If care is not taken to establish the validity of samples collected in the field and transported to a laboratory for analysis, potential evidence for a court action may be lost or ruled invalid.

7.2.5.2 Several types of evidence including oral and hearsay, circumstantial, and graphic may be collected during an investigation. Oral and hearsay evidence should be signed and dated by the individual giving the information. Circumstantial evidence must be carefully documented as to methods of collection, who collected it, and disposition of the evidence. Graphic evidence such as photographs should be accompanied by data listing when taken, how, by whom, the type of camera and film used, and who processed the film.

7.2.5.3 All samples must be handled in a similar orderly procedure and a complete record should be kept on their disposition. Recognized tests should be used and such tests must be approved in detail by USEPA or other recognized authorities. New test methods must be technically defensible. All unused portions of samples must be saved until released by the USEPA attorney working on the case.

7.2.5.4 The investigative team should make every effort to educate the attorney handling the case. The attorney should be aware of the expertise of the team, the methods used, validity of evidence collected, and complete disposition of the evidence.

TABLE 1. FLOWCHART FOR THE COORDINATION OF A FISH KILL INVESTIGATION[1]



[1]Modified from Meyer and Barclay (1990). Abbreviations: U.S. Environmental Protection Agency (USEPA), U.S. Coast Guard (USCG).

7.2.6 Field Investigations

7.2.6.1 The following is a brief discussion of a suggested method of field investigation (Meyer and Barclay (1990) provide guidelines, detailed, and specific procedures for fish kill field investigation). For additional methods, see the following references: AMPA (1992), Amer. Fish. Soc. (1982), ASTM (1992), Burdick (1965), Hill (1983), Smith et al. (1956), Tracy and Bernhardt (1972), U.S. Dept. Interior (1970), USEPA (1973), USEPA (1979a,b), USEPA (1980), and Section 12, Fisheries Bibliography, Subsection 12.7 Fish Kills.

7.2.6.2 Individuals involved in fish kill investigations should have a copy or be familiar with the document, *Field Manual for the Investigation of Fish Kills*, (F.P. Meyer and L.A. Barclay, eds., 1990). This document contains detailed information on the following: planning the investigation (Hunn, 1990), interpreting the fish kill location (Meyer and Herman, 1990), toxic substances effects and diagnosis (Hunn and Schnick, 1990), fish kills due to natural causes (Herman and Meyer, 1990), role of infectious agents in fish kills (Herman, 1990), quality assurance and legal requirements (Schnick, 1990a), where to send samples for analyses (Schnick, 1990b), shipping samples (Barclay, 1990a), writing the fish kill report (Meyer, 1990a), preparing for legal testimony (Barclay, 1990b), specific equipment needed for field assessments (Ardinger, 1990), and case histories of fish kills (Meyer, 1990b).

7.2.6.3 Since the speed with which an investigative team arrives at a fish kill is extremely important, a few advanced preparations are necessary. The public should be aware of whom to contact and where to report fish kills. If possible, a Region- or State-wide network of designated fish kill investigators should be established, each representing an area in which an investigator knows the water, biota, and potential polluters. In preparation for quick action, an investigator must have at his/her immediate disposal: telephone report sheets (Table 2), a checklist of equipment items (Table 3), maps of the area, and a list of cooperating analytical laboratories.

7.2.6.4 Make a reconnaissance of the kill area. Make a decision as to the extent of the kill and if a legitimate kill really has occurred. If a legitimate kill exists take steps to trace or determine the cause. Secure sampling equipment and determine size of investigative team needed. Standard equipment should be taken on all investigations (Table 3), and a standard checklist with space for special equipment will often save embarrassment in the field. The on-site study includes specific field observations (Table 4) that may be made on a fish kill form (Table 5). In addition, specific field observations (Table 4) should be emphasized, and complete weather data should be collected (for the period) prior to and during the fish kill. Water conditions both in and outside the affected area should be noted (i.e., appearance of water, turbidity, algal blooms, oil, unusual appearance, etc.). Stream flow patterns (i.e., high or low flow, stagnant or rapidly moving water, tide moving in or out, etc.) should be noted and recorded. If possible, obtain discharge reading from stream gauge if one is near fish kill area. During the initial steps of the investigation, water chemistry and physical parameters (e.g., pH, dissolved oxygen, temperature, specific conductance, and flow) must be determined immediately upon arrival at the kill site. While none of these factors may be directly involved in the fish kill, these tests are simply and rapidly performed in the field and can be used as a baseline for isolating the cause(s) of the kill. Make a rough sketch or define the fish kill area on a map so that sampling points, sewer outfalls, etc. can be accurately located on a drawing to be included in a final report. Take close-up and distance photographs of the dead fish in the stream in the polluted area, the stream above the polluted area, and the wastewater discharges. Photographs will often show a marked delineation between the wastewater discharge and the natural flow of water. Pictures taken at a relatively high elevation, (a bridge as opposed to a boat or from a low river bank) will show more and be more effective. Color photographs are also more effective than black and white prints in showing physical conditions of a stream.

7.2.6.5 Certain biological observations should also be made as soon as possible: (1) the presence or absence of plankton blooms, (2) dead or living macroinvertebrates and fish, and (3) the actions of moribund fish. Additional observations are listed in Tables 6, 7, and 8.

7.2.6.6 The location of sampling stations is very important. If there are no obvious reasons for a kill, stations should be selected in and outside the apparent kill area. If there are possible polluters, each should be suspect and sampling stations must be selected within and outside of the area of influence for each possible suspect.

7.2.6.7 In flowing waters, where a pollutant may be discharged as a slug, the investigator should try to estimate the time of kill, determine stream velocity, and collect samples downstream in the vicinity of the slug.

7.2.6.8 Water samples must be collected and processed in a variety of ways depending on the types of analyses required. An updated USEPA methods list for collection and preservation of samples should be at the disposal of the investigator (USEPA, 1979a,b).

7.2.6.9 The collection and preservation of aquatic organisms may require special techniques. For example, it is always best, if possible, to collect moribund fish from the affected area. If none are available, freshly dead fish will have to be utilized. Unaffected fish from outside the kill area must also be collected. All samples should be handled with regard to the type of suspected toxicant and the type of analysis to be performed.

7.2.6.10 Contact personnel from the laboratory or laboratories which will participate in analyzing samples. If possible estimate the following and record on the fish kill general information form (Table 2).

1. The number and size of samples to be submitted.

2. The probable number and types of analyses required.

3. The dates the samples will be received by the laboratory.

4. Method of shipment to the laboratory.

TABLE 2. FISH KILL GENERAL INFORMATION FORM

1. Who is the informant?

   Name ______________________________ Phone ______________

   Address ____

   ____

   Directions to meeting place ______________________________

   ____

   Date and Time ____

2. Reporting Source

   Agency ____

   Address ____

   Phone(s) ____

   Fish Kill Network __________________ Yes ________ NO ________

3. Location of kill (county, town, access point): __________________

   ____

4. Duration of kill: First noticed - Time ________ Date ______________

   Is it continuing? (Yes, No). If not, when did it stop ______________

5. Extent of Kill: Area covered (miles of stream or size of pond or lake).

   ____

6. Approximate number of fish affected _______ Species ______________

   ____

   ____

   Size (length, age classes) ________________________________

   ____

   ____

TABLE 2. FISH KILL GENERAL INFORMATION FORM (CONTINUED)

7. Opinion as to cause ______________________________

____

____

____

8. Recent activities (crop dusting, weather change, etc.) ______________

____

____

9. Possible sources of pollution ______________________________

____

____

10. Measures taken ______________________________

11. Action Requested

Field Investigation ______________________________

Laboratory Analyses ______________________________

12. Assistance to Project

Provided by ______________________________

Personnel ______________________________

____

Equipment ______________________________

____

Transportation Facilities ______________________________

____

____

____

TABLE 3. CHECKLIST OF FISH KILL INVESTIGATION EQUIPMENT

General

1. Boat
2. Motor
3. Paddles
4. Life preservers
5. Camera
6. Film
7. Ice chests
8. Wet ice
9. Dry ice
10. Portable light source
11. Waterproof notebook
12. Waterproof pencils
13. Waterproof labels
14. Chain of custody seals
15. Chain of custody forms
16. Arm-length gloves
17. Hip boots
18. Chest waders
19. Paper towels
20. Aluminum foil
21. Thermometer
22. Plastic bags, assorted sizes
23. DO kit
24. pH equipment (probe, colorimeter)
25. Glass jars (chemical samples)
26. Maps
27. Hand tallys
28. Tape measure (100 yd. or meter)
29. Rain gear
30. Polaroid glasses
31. Tamper proof seals

Plankton-Periphyhton

1. Water sampler - Van Dorn
2. Vials, small widemouth jars
3. 6-3-1, Formalin preservative
4. 2-liter jars

Fish

1. Dipnets
2. Seines
3. Nets
4. Electrofishing gear (if available)
5. Weight scale
6. Measuring board
7. Tubs
8. Fish counting forms
9. Dissecting kit
10. Heparinized vials
11. 10% formalin
12. 70-75% ethyl alcohol (ethanol)
13. Scale envelopes

Benthos

1. Ekman grab sampler
2. Ponar grab sampler
3. Surber-type sampler
4. Drift net sampler
5. Dipnets, kick nets
6. Quart and pint widemouth containers
7. 70-75% alcohol
8. 10% formalin
9. Foot tub
10. U.S. Standard 30 sieve
11. Forceps

TABLE 4. FIELD OBSERVATIONS

1. Locate the kill area.

2. Take water samples for chemical analysis and preserve properly.

3. Make chemical and physical field analyses (DO, pH, temperature, flow, weather, etc.).

4. Record observations (odors, flocks, sheens, deposits, etc.).

5. Collect fish for analyses (follow guidelines for various analyses).

6. Collect plankton samples.

7. Collect periphyton.

8. Collect macroinvertebrates (substrate, drifting, and attached).

9. Extensive and pertinent observations:

    * Observe and examine dead and dying fish (see other attachment.

    * Are small fish collected in tributaries--on surface or not?

    * Are the plankters (planktonic organisms) concentrated in the kill area?

    * Are they alive and viable or dead?

    * Is there extensive periphytic growth?

    * Is the benthic community active, over-active, quiescent?

    * Are there many drifting organisms?

    * Record all observations.

10. Repeat the applicable steps above in a non-affected area of the lake or stream.

11. Take numerous pictures of the overall area, specific problem areas, dying fish, algae blooms, water conditions (color, turbidity, etc.).

12. Counts of mortality by species to estimate resource loss.

TABLE 5. FISH KILL INVESTIGATION FORM

Stream, Lake, Other ____________ Drainage Basin ____________

Stream Mile ____________ Tributary to ____________

County ____________ State ____________ Top. Map. ____________

Nearest Town ____________ Highways ____________

Fish Kill Began: Time ________ Date ________ Ended: Time ________ Date ______

Time and Date of First Report ____________ Reported by (Name) ____________

Address ____________ Telephone ____________

Investigators: (Name and Agency) ____________

____

Area Affected: Upstream Limits ____________
Downstream ____________
Miles ____________ Acres ____________

Weather Conditions: Present ____________
Past 48 Hours ____________

Photographic Record:

Picture No.	Time and Date	Subject

Field Measurements:

Sample	Upstream of Kill	In Kill Area	Downstream of Kill
Temp.			
pH			
DO			
Conductivity			
Gage Ht./Flow			

Comments and Possible Sources:

TABLE 5. FISH KILL INVESTIGATION FORM (CONTINUED)

Location of Collected Samples

Sample ID. No.	Station Description

Sample ID No:

Sample	Upstream of Kill	In Kill Area	Downstream of Kill
Water Sample*			
Fish (frozen)			
Fish (formalin)			
Fish (fresh)			
Fish Blood:			
Species length			
Species length			
Species length			
Species length			
Sediment			
Algae (frozen)			
Algae (iced)			
Benthos			
Special Analysis			

Approximate # of dead fish of (of each species)/acre, mile, 100 yards, etc.

*Requested Analyses:

TABLE 6. OBSERVATION ON DEAD AND MORIBUND FISH

External

1. External examination for fungus, bacteria, open sores, parasites.
2. Gill examination for color, abnormal morphology, gill lice, slime, collapse of filaments, adhesion of filaments.
3. Eyes opaque, clear, covered by mucus.
4. Fins - anchor lice, extended-folded, bleeding, fungused, frayed.
5. Scales - loose groups, bent, bleeding, missing.
6. Body - bent, twisted, rigid.
7. Mouth - open, normal, hyper-extended in death.

Internal

1. Do they bleed freely?
2. Is the liver clear of spots or open lesions? Is it a light off-brown or tan?
3. Is the air bladder hard, very soft, or partly inflated?
4. Is the stomach full or empty? What is in it?
5. Is the entire intestinal tract empty?
6. Are there internal parasites in the abdominal cavity?
7. Is there watery fluid in the abdomen?
8. Is there discoloration of any of the tissues?
9. Are the muscles pulled away from the ribs or backbone?
10. Are there lesions or spots in the muscles? Describe them.
11. Is the kidney (against the backbone) a normal dark red to purple or unspotted?
12. Are there lesions or watery abscesses (i.e., blisters)?
13. Is the pericardial space free with watery fluid or is it discolored a reddish or yellow color?
14. Are the fish slimy or dry?

TABLE 6. OBSERVATION ON DEAD AND MORIBUND FISH (CONTINUED)

Internal (continued)

15. Are there trailing mucus strings from the gills or fins?

16. Are there large patches of missing scales?

17. Is there bleeding about the fin bases or scale bases?

18. Do the gills look very bright red, dark blue, or purple? Are the gills covered with slime? Are they bleeding or lumpy?

19. Do the gill covers move very rapidly or very slowly?

20. Are the fish unresponsive, roll over in the water, and slowly die? Do they slowly settle to the bottom while upright?

21. Do any rest upside down at the surface and still breathe?

22. Do any cough, flare the gill covers, or flare the fins?

TABLE 7. OBSERVATIONS ON EFFECTED FISH

1. Do the fish swim wildly at the surface? If they do, do they do it continuously or in erratic and irregular bursts of activity?
2. Do they try to leap from the water after racing across the surface?
3. After they race at the surface, do they fall on their side and tremble?
4. How long do they race about?
5. Do they race about, then tremble, turn over, and die , or do they race, rest, then race with increasing periods between bursts of activity?
6. At the end of a run, are the bodies twisted or rigidly bent to one side or the other?
7. As activity decreases, do they rest upright at the surface?
8. As activity decreases, do they rest head-down in the water?
9. As activity decreases, do they rest tail-down in the water?
10. As activity decreases, do they rest tail-down in the water and spin on their long axis?
11. With the slower erratic swimming, do they swim forward, slowly turning over and over, spiraling, or swim forward but describe a long curving arc or circle?
12. Do they swim slowly forward, mouthing at the surface with audible "smacking" sounds?
13. Do they swim slowly forward, ejecting bubbles from the mouth?
14. As swimming slows or ceases, do they settle into the water or do they struggle to stay down and upright?
15. If you can catch them, must you use a net, or can you catch them by hand?
16. Once caught, do they struggle, tremble, lose scales, or go rigid?
17. Are they bleached out, very dark, or blotchy?
18. Are there fuzzy blotches anywhere on the body?
19. Are there open scores?
20. Are the fins and gill covers folded or held rapidly extended from the body?

TABLE 7. OBSERVATIONS ON EFFECTED FISH (CONTINUED)

21. Are they slimy or dry?
22. Are there trailing mucus strings from the gills and fins?
23. Are there large patches of missing scales?
24. Is there bleeding about the fin bases or scale bases?
25. Do the gills look very bright red, dark blue, or purple? Are the gills covered with slime? Are they bleeding or lumpy?
26. Do the gill covers move very rapidly or very slowly?
27. Are the fish unresponsive, roll over in the water, and slowly die?
28. Do they slowly settle to the bottom while upright.
29. Do any rest upside down at the surface and still breathe?
30. Do any cough, flare the gill covers, or flare the fins?

TABLE 8. SYMPTOMS THAT HAVE BEEN RELATED TO CAUSE OF FISH DEATH[1]

SYMPTOM	CAUSATIVE AGENTS
Gasping at surface	Low DO or rotenone
Fish dying in early morning only	Low DO, summer kill
Swimming slowly in circles or only one species affected	Disease
Erratic swimming patterns, contorted bodies, tremors, or convulsions. Other animals involved (i.e., birds,snakes, turtles, etc.)	Pesticides
Fish gills covered with mucus, or clogged	Rotenone, high suspended solids, heavy metals
Small fish kills of various species over a long period of time, altered species composition	Low concentrations of trace metals
Deflated swim bladders and viscera obliterated	Seismic blasts, dynamite, or other explosives.
White film on gills, skin and mouth	Acids, heavy metals, trinitrophenol
Sloughing of gill epithelium	Copper, zinc, lead, detergent, ammonia, quinoline
Gill occlusion	Turbidity, ferric hydroxide precipitate
Bright red gills	Cyanide
Dark gills	Phenolic poisoning, p-cresol, naphthalene, oxygen deficiency
Gill lamellae thickening	Hydrogen sulfide
Distended gill covers	Ammonia, cyanide

[1]Modified from Janet Kuelfer, USEPA, Region 9, San Francisco, CA.

TABLE 8. SYMPTOMS THAT HAVE BEEN RELATED TO CAUSE OF FISH DEATH (CONTINUED)

SYMPTOM	CAUSATIVE AGENTS
Swollen abdomens	Chlorinated hydrocarbon, insecticides
Blue stomachs	Molybdenum
Intestinal epithelium destruction	Hexavalent chromium, pulp mill wastes
Gall bladder distension	Pulp mill wastes
Extreme thinning of stomach wall	Endosulfan
Pin point white spots, fish rubbing against substrate	*Ichthyophonus* sp., *Cryptocaryon* sp. (Ich disease)

5. To whom the laboratory results are to be reported.

6. The date the results are needed.

7.2.7 General Sampling Procedures (also see Meyer and Barclay, 1990). The extent and method of sampling will depend upon location and upon the suspected cause of the kill.

7.2.7.1 For stream and wastewater sampling, sample the following points when the pollution discharge is coming from a well defined outfall:

1. The effluent discharge outfall.

2. The stream at the closet point above the outfall which is not influenced by the waste discharge.

3. The stream, immediately below the outfall.

4. Other points downstream needed to trace the extent of the pollution.

7.2.7.2 The sampling should be extensive enough that when all the data is compiled no question will exist as to the source of the pollution which killed the fish.

1. Streams less than 200 feet wide, not in an industrial area usually can be adequately sampled at one point in a section (Figure 1).

2. Streams 200 feet or wider generally should be sampled two or more places in a section immediately above and below the pollution discharge. Where the pollutional waste has adequately mixed with the stream flow one sample may suffice.

3. A number of samples in a cross section may be required on any size of stream to show that the suspected pollutional discharge is coming from a source located in an industrial or municipal complex (Figure 2).

4. Extensive cross sectional sampling on rivers greater than 2000 feet wide will be required for kills involving suspected agricultural or other types of mass runoff.

5. Sample depth - on streams 5 feet in depth or less, one mid-depth sample per sampling location is sufficient. For streams of greater depths, appropriate sampling judgment should be used since stratification may be present.

7.2.7.3 The number of samples to be collected at a given cross section will depend principally on the size of the stream.

   a. Ten 1 L water samples should be collected from the kill area for chemical analyses as well as other 1 L samples from control and other stations. (In flowing waters samples should also be collected in the estimated location of the main slug).

b. Ten pounds including ten individuals of dying fish of each important species frozen with dry ice. An equivalent amount and number of control fish.

c. Five small fish of each important species preserved in formalin.

d. Five dying fish of each significant species placed on wet ice and delivered to a fish disease laboratory within 24 hours. (Some fish disease labs specify fish placed in bags next to wet ice.)

e. A minimum of ten fish should be collected for histochemical analysis. Refer to Section 5, Fish Specimen Processing, on the proper fixation and preservation of fish tissues for histochemistry methods.

f. Five vials containing 5 cc. each of blood from each important species.

g. Ten gallons of water for bioassay.

h. One quart to one gallon of sludge or sediment.

i. Ten cc. of concentrated algae frozen.

j. Ten cc. of concentrated algae chilled.

k. Benthic invertebrate (macroinvertebrates) samples.

7.2.8 Explanation of Figures 1 and 2.

7.2.8.1 Collection point 1 (Figure 1) and points 3 and 4 (Figure 2) should be collected as near to the point of pollutional discharge as possible. These points will vary according to stream flow conditions. The pollution discharges into a slow sluggish stream usually will have a cone of influence upstream of the outfall; whereas, a swift flowing stream usually will not.

7.2.8.2 Collecting an upstream control sample from a bridge within sight of the pollutional discharge would probably be satisfactory in Figure 1 but definitely not in Figure 2.

7.2.8.3 Figures 1 and 2 are given for illustrative purposes only and should be used only as a guide for sampling. Each individual situation must be individually considered to insure adequate, proper sampling. While too many samples are better than too few, effort should be made not to unduly overload the laboratory with samples collected as a result of poor sampling procedures.

7.2.9 Biological Sampling

7.2.9.1 In every investigation of fish kills the paramount item should be the immediate collection of the dying or only recently dead organisms. Sampling and preservation are as follows:

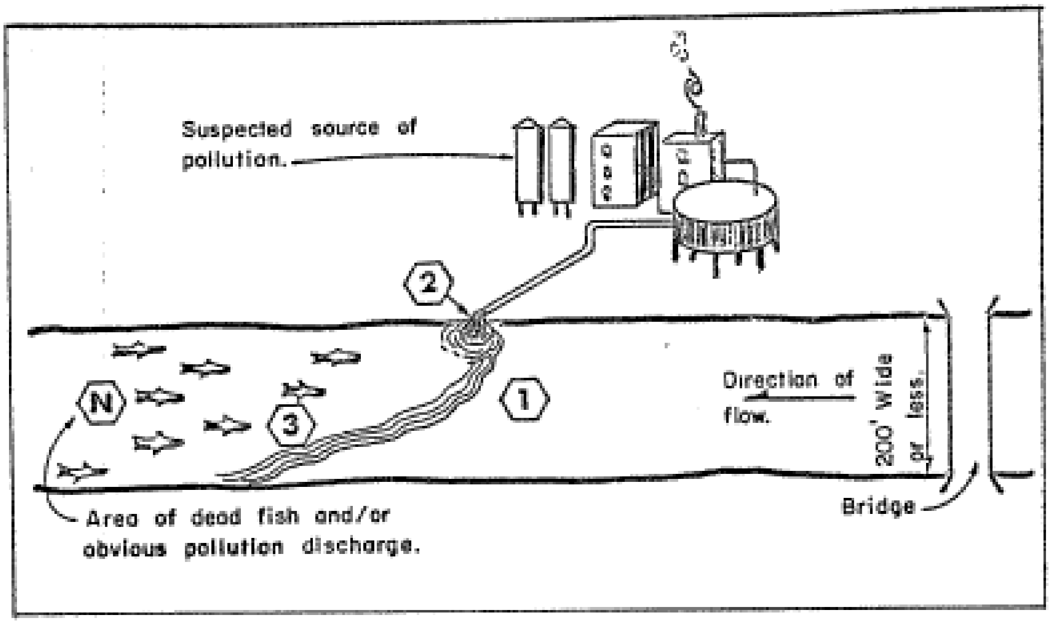


Figure 1. Minimum water sampling point on stream 200 feet or less wide involving an isolated discharge. Modified from USEPA (1973).

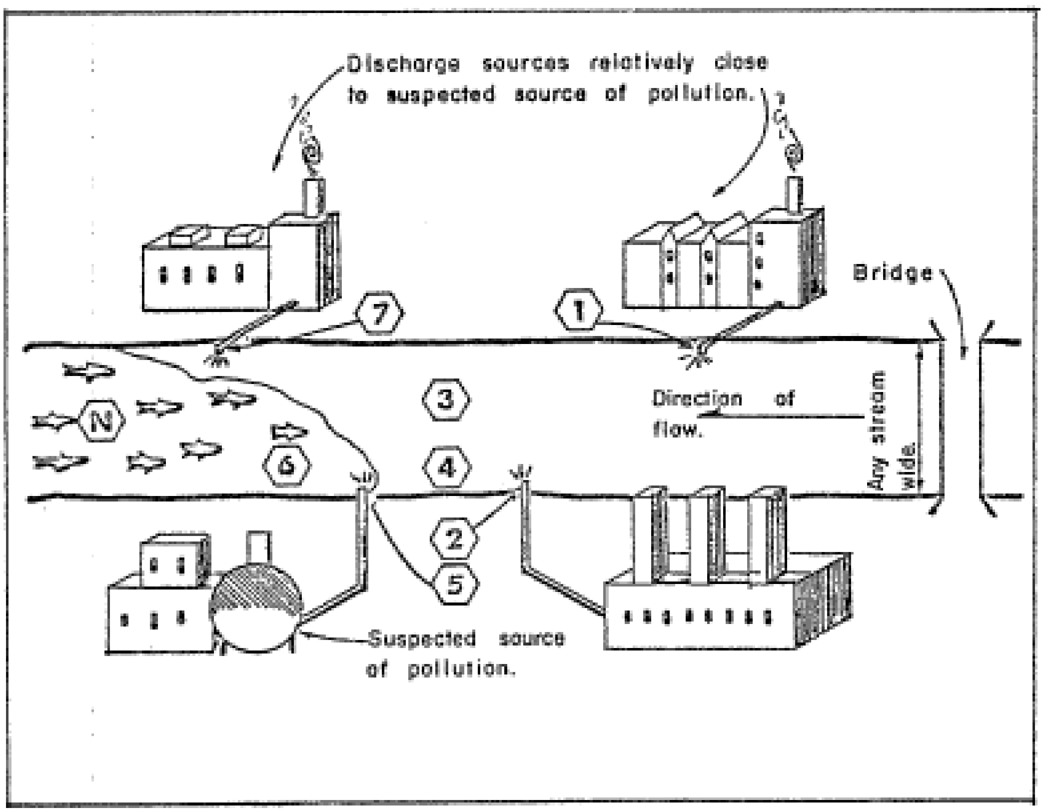


Figure 2. Minimum water sampling points on a stream running through an industrial or municipal complex. Modified from USEPA (1973).

1. Collect 20+ drops of blood in a solvent rinsed vial, seal with aluminum foil, cap, and freeze.

2. Place bleeding specimens, or entire specimens if beyond bleeding stage, in plastic bag and freeze. In case no method of freezing is available, icing for a short period prior to freezing may be acceptable. Labeling of both blood and carcass is important.

3. Controls - live specimens of the affected organisms should be obtained from an area within the same body of water which had not been influenced by the causative agent. Once obtained these specimens should be handled in a like manner.

7.2.8.2 The number of individuals involved and the species affected should be enumerated in some manner. At most these will be estimates. Depending on the given situation such as area or distance involved and personnel available, enumeration of fish kills may be approached in one of the following ways:

1. For large rivers, establish observers at a station or stations (e.g., bridges) and count the dead and/or dying fish for a specified period of time, then project to total time involved.

2. For large rivers and lakes, traverse a measured distance of shoreline, count the number and kinds of dead or dying fish. Project numbers relative to total distance of kill.

3. For lakes and large ponds, count the number and species within measured areas, and then project to total area involved.

4. For smaller streams one may walk the entire stretch involved and count number of dead individuals by species.

7.2.9 Sampling Other Biota

7.2.9.1 Sampling of benthic organisms after the more urgent aspects of the kill investigation has been completed can prove to be valuable relative to the extent and cause of the kill. Benthic invertebrate communities are sampled to determine whether this assemblage, the primary food source of many fishes, has been affected. Also, since this general form of aquatic life is somewhat sedentary by nature, release of deleterious materials to their environment will kill much of the biota. By making a series of collections up and downstream from the affected area, the affected stretch of stream may be delineated when the benthic populations are compared to those organisms from the control area. Also, the causative agent may be realized when the specifics of the benthic population present are analyzed. Other aspects of the biota which should be considered are the aquatic plants. In lakes and ponds floating and rooted plants should be enumerated and identified. The collection of plankton samples (river and lakes) should be taken in order to determine possible toxicity from toxin-producing species and to determine the degree of bloom, which in itself may cause fish kills because of diurnal dissolved oxygen levels. Both aquatic plants and macroinvertebrates may be fixed in a 10% formalin solution and preserved in 70% ethanol.

7.2.9.2 When the material causing a kill is known, some of the above sample collections may not be required. However, if the cause of a kill is unknown, the above samples plus other specific samples, dictated by the type of fishery, may be required.

7.2.9.3 Graphic evidence has a maximum effect on people involved in pollution cases. Two basic types of graphic evidence are: (1) hand drawn maps of the general and specific location of the kill, extent of kill, plankton bloom, location of dead and dying fish, etc. (2) photograph (color and black-and-white) showing dead fish, oil slicks, nasty looking water, sampling location, etc. All graphic evidence should be carefully documented as recommended in Subsection 7.2.5, Legal Aspects, and Meyer and Barclay, 1990).

7.2.9.4 The magnitude of a fish kill should be carefully documented. A recognized method for enumerating the number and species which have been killed should be selected and carefully followed so that data collected will be admissible as evidence. Such methods are found in Meyer and Barclay (1990) and references cited in Subsections 7.2.6.1 and 7.2.6.2.

7.2.10 Bioassays

7.2.10.1 Static bioassay techniques, as outlined in USEPA (1991), may be effectively used to determine acute toxicity of wastes as well as receiving waters. Toxicity testing can be done in-situ using live boxes, a mobile bioassay laboratory, or the samples can be returned to a central laboratory for testing.

7.2.11 Report

7.2.11.1 The final report should contain accurate information and should be well organized to meet the requirements under Legal Aspects (Subsection7.2.5). Essential elements of the report are: (1) introduction, (2) summary, (3) description of the area, (4) description of all sampling methods and analyses, (4) discussion of the magnitude of the fish kill and effects on other aquatic organisms, (5) discussion of other water users in the affected area and (6) conclusion. For additional recommendations, see the references listed in Subsections 7.2.6.1, 7.2.6.2, and Section 12, Fisheries Bibliography, 12.7 Fish Kills.

7.2.12 Case History

7.2.12.1 A lower Mississippi River endrin-caused fish kill is an excellent example of the investigation of a major fish kill Bartsch and Ingram (1966) give the following summary (Table 9).

7.2.12.2 The investigation was designed to consider and eliminate potential fish kill possibilities that were not involved and come to a point focus on the real cause. It was found that the massive kills were not caused by disease, heavy metals, organic phosphorus compounds, lack of dissolved oxygen or unsuitable pH. Blood of dying river fish was found to have concentrations of endrin equal to or greater than laboratory fish killed with this pesticide, while living fish had lesser concentrations. Symptoms of both groups of dying

TABLE 9. SUMMARY OF A LOWER MISSISSIPPI RIVER ENDRIN FISH KILL INVESTIGATION[1,2]

I. Examination of usual environmental factors.

II. Elimination of parasites, bacterial or viral diseases, and botulism as causes of mortalities[1].

III. Consideration of toxic substances: Examination and prognostication of symptoms of dying fish. Necropsy including:

Haematocrits and white cell counts

Brain tissue assay for organic phosphorus insecticide

Kidney tissue study

Tissue analysis for 19 potentially toxic metals

Gas chromatographic analysis of tissues, including blood, for chlorinated hydrocarbon insecticides

IV. Exploration for toxic substances:

Bioassay with Mississippi River water

Bioassay with extracts from river bottom mud

Bioassay with tissue extracts from fish dying in river water and bottom mud extracts

Bioassay with endrin to compare symptoms and tissue extract analyses with those of dying fish in all bioassays.

V. Intensive chemical analysis for pesticides in the natural environment, experimental environment, river fish, and experimental animals.

VI. Surveillance of surface waters for geographic range and intensity of pesticide contamination.

VII. Correlation and interrelation of findings.

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[1]Modified from Bartsch and Ingram (1966).
[2]The investigator should be aware of the fact that apparently healthy fish may be harboring pathogenic bacteria in their bloodstreams (see Bullock and Snieszko, 1969). Thus, there may be several factors involved in fish mortalities, all of which may obscure the primary cause or causes.

fish (river and bioassay) in the study (Table 9) were identical. It was concluded from all data obtained that these fish kills were caused by endrin poisoning.

7.3 Instream Flow Incremental Methodology (IFIM)

7.3.1 The IFIM was developed by Bovee (1982) for the U.S. Fish and Wildlife Service and is widely utilized in the United States by the U.S. Fish and Wildlife Service, state agencies, and consulting firms to estimate the effect of change in instream flow on the habitat of stream fish and other aquatic organisms (Baldridge and Amos, 1981; Gan and McMahon, 1990; Gore and Judy, 1981; Hilgert, 1982; Irvine et al., 1987; Mathur et al., 1985; Orth and Maughan, 1982, 1986; Parsons and Hubert, 1988; Waite, 1989; Waite and Barnhart, 1992). This methodology is only discussed here generally, but investigators should consult the authors cited in this Subsection for their application of fisheries bioassessment, management, and related research needs.

7.3.2 The application of the IFIM and its effectiveness have been evaluated and reviewed by several researchers (Bayha, 1978; Conder and Annear, 1987; Gan and McMahon, 1990; Gore and Nestler, 1988; Irvine, et al., 1987; Mathur et al., 1985; Orth and Maughan, 1982; 1986; Shirvell, 1989; Waite, 1989; Waite and Barnhart, 1992). In addition, Wesche and Rechard (1980) reviewed and summarized instream flow methods for fisheries and related research needs.

7.3.3 An important element of the IFIM is the use of physical habitat simulation (PHABSIM) computer models (e.g., IFG-4, HABTAT) that relate changes in discharge or stream channel structure to changes in the availability of physical habitat (Waite and Barnhart, 1992). With PHABSIM the hydraulic and physical variables of a stream or river are simulated for an assigned flow, and the amount of usable habitat (weighted usable area or WUA) can be predicted for a particular life stage of a particular species of fish. The prediction of WUA is based on ecological data and on habitat use by selected species of fish at various developmental life stages. The data are expressed in terms of habitat utilization or probability of use curves (Bovee and Cochnauer, 1977; Raleigh et al., 1984). The habitat utilization curves most commonly used in the IFIM are those for current velocity, substrate particle size, and water depth. According to Parsons and Hubert (1988), the values that are generated by an IFIM study can be misleading if the habitat utilization curves do not adequately reflect the conditions that fish of a life stage need, prefer, or tolerate. In addition, the type of habitat used by stream salmonids varies by species, life stage of the species, and characteristics of the available habitat. Using data found in the literature and additional research, Bovee (1978), Bovee and Cochnauer (1977), and Raleigh et al. (1984) compiled and developed general standard habitat utilization curves which could be broadly applied. Shirvell (1989) found that generic curves were not always accurate. Waite and Barnhart (1992) developed habitat utilization curves for allopatric fry and juveniles of steelhead *Oncorhynchus mykiss* over a range of environmental conditions in a small stream with moderate to high gradient, and also compared these curves with three standard IFIM probability of use curves. Waite and Barnhart (1992) also concluded that applying habitat utilization curves of one stream to generate WUA values for a different stream should be done only after the investigator has measured and compared other stream characteristics, such as stream width, flow, gradient, depth, substrate particle size, pool:riffle ratio, and seasonal hydrography.

7.3.4 Recent studies by Layher and Brunson (1992) involve modification of the habitat evaluation procedures for determining instream flow requirements in warmwater streams; Olson-Rutz and Marlow (1992) studied the analysis and interpretation of stream channel cross-sectional data and discussed stream channel form and bank stability importance to the biotic community structure of riparian ecosystems.

7.4 Fish Marking and Tagging Techniques (Mark-and-Recapture)

7.4.1 The marking and tagging of fish are important techniques utilized to obtain information necessary for research and management. They are often used to study individual fish or fish populations. Marking or Tagging studies can give investigators data on estimates of biomass, stocking success, migrations, behavior, age, mortality rates, etc. For a review and synthesis of the different types of devices and techniques (e.g., external, internal, electronic, genetic, chemical tags and marks, etc.), consult Lagler (1956, 1978), Wydoski and Emery (1983), Parker et al. (1990).

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